

USSN 09/308,207  
Page 8

### REMARKS

With entry of the present amendment, claims 19 – 21, 26 – 31 and 41 - 51 are pending. Claims 19, 20, 26 - 28, 43 - 45 and 47 - 50 have been amended. New matter has not been introduced by the amendment to the claims. Claims 1 – 18, 22 – 25 and 32 – 40 were previously canceled. New claims have not been added. Pending claims 19, 47 and 49 are independent claims. For the Examiner's convenience, Applicants have included, as an appendix hereto, a clean unmarked copy of the pending claims.

Independent claims 19, 47 and 49 have been amended to recite that production of 1,3 propanediol from the recombinant microorganism including a gene encoding protein x is greater than in a recombinant microorganism lacking said gene. Further the claims have been amended to define the gene encoding a glycerol dehydratase as obtained from *Klebsiella* or *Citrobacter* and a gene encoding a diol dehydratase as obtained from *Klebsiella*, *Clostridium* or *Salmonella*. In addition, claim 19 has been amended to delete element b) directed to a gene encoding a glycerol-3-phosphatase. This element has been incorporated into dependent and currently amended claim 43.

Claims 20, 48 and 50 have been amended to recite specific sequence identifiers for protein 1, protein 2 and protein 3.

Claims 28 and 49 have been corrected to recite a nucleic acid sequence of residues 9749 - 11572 of SEQ ID NO:19.

The term "activity" has been eliminated from claims 26, 27 and 44. Claim 45 has been amended to recite the amino acid sequence of SEQ ID NO: 67.

### Objections -

Applicants have amended the specification to change the title of Example 1 to include the phrase, "production of 1,3-propanediol". The address of ATCC has been corrected to the current address. Applicants have amended the description of Figures 2A – 2G to reflect that the nucleotide sequence of protein X is set forth in SEQ ID NO: 68 and the amino acid sequence is set forth in SEQ ID NO: 59. However, Applicants are confused by the Examiner's statement,

GC369-2A-USAM 09/04

USSN 09/308,207  
Page 9

"Protein X encoded by gene dhab4 as presented in Figure 2 is not that of SEQ ID NO: 59. Although at least 20 N-terminal and at least 20 C-terminal amino acids of both sequences are identical, the protein set forth by SEQ ID NO: 59 consist of 607 amino acids, whereas the protein DHAB4 whose amino acid sequence is presented Figure 2 consists of 727 amino acid."

Applicants believe the sequences as presented in the figure and in SEQ ID NO: 59 are the same. They both have 607 amino acids and this has been confirmed by one of the co-inventors.

The Examiner indicated that the ATCC numbers, 69789 and 69790 quoted on page 9, lines 30 and 33 of the specification were not valid and that correct ATCC numbers should be provided. (These numbers are also quoted at page 11, lines 15 – 21). Applicants submit that both ATCC designations are correct. Designation ATCC 69790 includes the plasmid pKP4, which includes the diol dehydratase from *K. pneumoniae* in *E. coli* as disclosed on page 9. This plasmid is available from ATCC. Additionally, ATCC 69789 includes the plasmid pKP1, which includes the glycerol dehydratase in *E. coli*. However, it appears that this deposit is currently unavailable from the ATCC.

Rejection under 35 U.S.C. §112, second paragraph.

Applicants acknowledge the withdrawal of rejections previously made under 35 U.S.C. §112, second paragraph of claims 19 - 31.

Presently claims 19 - 21, 26 - 31 and 41 - 51 have been rejected under 35 U.S.C. §112, second paragraph.

The Examiner has rejected claims 28 and 49 because the number "0749" in the phrase "as shown between positions 0749 - 11572 of SEQ ID NO: 19" was inadvertently mistyped. Correction has now been made to the claims which correctly reflect a nucleic acid sequence of residues 9749 - 11572 of SEQ ID NO: 19 and reference is made to page 11, lines 22 - 26.

The amino acid sequence referred to in claim 45 was incorrectly recited as SEQ ID NO: 66. As the Examiner points out, SEQ ID NO: 66 is a nucleotide sequence. This has been corrected.

USSN 09/308,207  
Page 10

Independent claims 19, 47 and 49 have been amended to clarify that the increased production of 1,3-propanediol in the recombinant microorganism is due to the introduction of the nucleic acid encoding protein X.

Applicants contend the present amendment to the claims renders moot each of the stated rejections under the second paragraph of section 112 and withdrawal of the rejections is requested.

Rejection under 35 U.S.C. §112, first paragraph.

The Examiner has alleged claims 19 - 21, 26 - 31 and 41 - 51 fail to comply with the written description requirement and enablement requirement of section 112.

With respect to the written description requirement the Examiner has said that the claims are directed to "a )any gene encoding a glycerol or diol dehydratase activity; b) any gene encoding a glycerol-3-phosphatase and c) gene encoding protein X wherein the gene is isolated from a glycerol dehydratase gene cluster of *Klebsiella* and *Citrobacter* or isolated from a diol dehydratase gene cluster of *Klebsiella*, *Clostridium* or *Salmonella*.

Applicants contend the written description requirement is fulfilled and the claims as amended are directed to a) genes encoding a glycerol dehydratase isolated from a glycerol dehydratase gene cluster of *Klebsiella* or *Citrobacter* or diol dehydratase isolated from a diol dehydratase gene cluster from *Klebsiella*, *Clostridium* or *Salmonella*; As stated above element b) as been cancelled from the independent claims.

The claims directed to genes encoding "protein 1, "protein 2" and protein 3" have been amended to recite specific sequences.

With regard to claims 28 and 45, there seems to be some confusion. The nucleotides of the gene (nucleotides corresponding to residue positions 9749 - 11572) encoding protein X, consists of 1823 nucleotides and not 1724, as suggested by the Examiner. A polypeptide encoded by 1823 nucleotides is 607 amino acids. Moreover as previously discussed, protein X of SEQ ID NO: 59 is coded for by the gene dhab4 as disclosed in Figure 2A-2G and SEQ I NO 68.

Applicants also submit that the claims are enabled and while small routine screening may be required, undue experimentation would not be required because the specification provides a reasonable amount of guidance with respect the claim invention. The claims as amended recited specific sequences for protein 1, protein 2 and protein3,

GC369-2A-USAM 09/04


USSN 09/308,207  
Page 11

and further genes encoding a glycerol dehydratase or a diol dehydratase must be obtained from specific organisms.

It is believed that the claims, as currently amended, are in condition for allowance and reconsideration is respectfully requested. If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 846-7620.

Respectfully submitted,

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GC369-2A-USAM 09/04

USSN 09/308,207  
Page 12

**APPENDIX –UNMARKED COPY OF PENDING CLAIMS**

19. (Currently amended): A recombinant microorganism capable of producing 1,3-propanediol from a carbon source said recombinant microorganism comprising

- a) at least one introduced gene encoding a glycerol dehydratase from *Klebsiella* or *Citrobacter* or a diol dehydratase from *Klebsiella*, *Clostridium* or *Salmonella* and
- b) at least one introduced gene encoding protein X, wherein the gene encoding protein X is i) isolated from a glycerol dehydratase gene cluster from an organism selected from the genera consisting of *Klebsiella* and *Citrobacter* or ii) isolated from a diol dehydratase gene cluster from an organism selected from the genera consisting of *Klebsiella*, *Clostridium* and *Salmonella* and wherein protein X has no enzymatic activity,

wherein production of 1,3-propanediol is greater in the recombinant microorganism comprising protein X than in the recombinant microorganism lacking said gene encoding protein X.

20. (Currently amended): The recombinant microorganism of Claim 19 further comprising d) at least one introduced gene encoding a protein selected from the group consisting of protein 1, protein 2 and protein 3, wherein said protein 1 has an amino acid sequence corresponding to SEQ ID NO:60 or SEQ ID NO:61; said protein 2 has an amino acid sequence corresponding to SEQ ID NO:62 or SEQ ID NO:63; and said protein 3 has an amino acid sequence corresponding to SEQ ID NO:64 or SEQ ID NO:65

21. (Previously presented): The recombinant microorganism of Claim 19, wherein the microorganism is selected from the group of genera consisting of *Citrobacter*, *Enterobacter*, *Clostridium*, *Klebsiella*, *Aerobacter*, *Lactobacillus*, *Aspergillus*, *Saccharomyces*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Pichia*, *Kluyveromyces*, *Candida*, *Hansenula*, *Debaryomyces*, *Mucor*, *Torulopsis*, *Methylobacter*, *Escherichia*, *Salmonella*, *Bacillus*, *Streptomyces* and *Pseudomonas*.

GC369-2A-USAM 09/04

USSN 09/308,207  
Page 13

26. (Original): The recombinant microorganism of Claim 19 wherein said dehydratase activity is heterologous to said microorganism.

27. (Original): The recombinant microorganism of Claim 19 wherein said dehydratase activity is homologous to said microorganism.

28. (Currently amended): The recombinant microorganism of Claim 19 wherein the gene encoding protein X consists of a nucleic acid sequence of residues 9749 - 11572 of SEQ ID NO:19.

29. (Original): The recombinant microorganism of Claim 20 wherein protein 1 has the sequence as shown in SEQ ID NO: 60 or SEQ ID NO: 61.

30. (Previously presented): The recombinant microorganism of Claim 20 wherein protein 2 has the sequence as shown in SEQ ID NO: 62 or SEQ ID NO: 63.

31. (Previously presented): The recombinant microorganism of Claim 20 wherein protein 3 has the sequence as shown in SEQ ID: 64 or SEQ ID NO: 65.

41. (Previously presented): The recombinant microorganism of Claim 19, wherein the carbon substrate is selected from the group of monosaccharides, oligosaccharides, polysaccharides and one-carbon substrates.

42. (Previously presented): The recombinant microorganism of Claim 21, wherein the recombinant microorganism is an *E. coli*, a *Klebsiella spp.* or a *Saccharomyces spp.*

43. (Previously presented): The recombinant microorganism of Claim 19, further comprising a gene encoding a glycerol-3-phosphatase selected from the group consisting of a nucleic acid molecule encoding the amino acid sequence of SEQ ID NO:17, SEQ ID NO:33, and enzymatically active fragments thereof.

44. (Previously presented): The recombinant microorganism of Claim 19, wherein the gene encoding the dehydratase is a glycerol dehydratase of *Klebsiella pneumoniae*.

GC369-2A-USAM 09/04

USSN 09/308,207  
Page 14

45. (Currently amended): The recombinant microorganism of Claim 19, wherein the gene encoding protein X is a nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 67.

46. (Previously presented): The recombinant microorganism of Claim 19, wherein protein X is encoded by the ORF Z of the *Citrobacter* dha regulon.

47. (Currently amended): A recombinant *E. coli* capable of producing 1,3-propanediol from a carbon source said *E. coli* comprising

a) at least one introduced gene encoding a glycerol dehydratase from *Klebsiella* or *Citrobacter* or a diol dehydratase from *Klebsiella*, *Clostridium* or *Salmonella*, and

c) at least one introduced gene encoding protein X,

wherein (i) protein X has no enzymatic activity, (ii) the gene encoding protein X is isolated from a glycerol dehydratase gene cluster from an organism selected from the genera consisting of *Klebsiella* and *Citrobacter*, or the gene encoding protein X is isolated from a diol dehydratase gene cluster from an organism selected from the genera consisting of *Klebsiella*, *Clostridium* and *Salmonella*, (iii) the carbon source is selected from the group of monosaccharides, oligosaccharides, polysaccharides and one-carbon substrates, and (iv) production of 1,3-propanediol is greater in the recombinant *E. coli* comprising protein X than in the recombinant *E. coli* lacking said gene encoding protein X.

48. (Currently amended): The *E. coli* of Claim 47 further comprising at least one introduced gene encoding a protein selected from the group consisting of protein 1, protein 2 and protein 3, wherein said protein 1 has an amino acid sequence corresponding to SEQ ID NO:60 or SEQ ID NO:61; said protein 2 has an amino acid sequence corresponding to SEQ ID NO:62 or SEQ ID NO:63; and said protein 3 has an amino acid sequence corresponding to SEQ ID NO:64 or SEQ ID NO:65.

49. (Currently amended): A recombinant microorganism capable of producing 1,3-propanediol from a carbon source said microorganism comprising

GC369-2A-USAM 09/04

USSN 09/308,207  
Page 15

a) at least one introduced gene encoding a glycerol dehydratase from *Klebsiella* or *Citrobacter* or a diol dehydratase from *Klebsiella*, *Clostridium* or *Salmonella*; and

b) at least one introduced gene encoding protein X, wherein the gene encoding protein X consists of a nucleic acid sequence of residues 9749 - 11572 of SEQ ID NO: 19; and

wherein production of 1,3-propanediol is greater in the recombinant microorganism comprising protein X than in the recombinant microorganism lacking said gene encoding protein X.

50. (Currently amended): The microorganism of Claim 49 further comprising at least one introduced gene encoding a protein selected from the group consisting of protein 1, protein 2 and protein 3, wherein said protein 1 has an amino acid sequence corresponding to SEQ ID NO:60 or SEQ ID NO:61; said protein 2 has an amino acid sequence corresponding to SEQ ID NO:62 or SEQ ID NO:63; and said protein 3 has an amino acid sequence corresponding to SEQ ID NO:64 or SEQ ID NO:65.

51. (Previously presented): The microorganism of Claim 49, wherein the recombinant microorganism is an *E. coli*, a *Klebsiella* spp. or a *Saccharomyces* spp.



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